Ultrasound in Med. & Biol., Vol. 34, No. 2, pp. 252–264, 2008

0301-5629/08/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2007.06.018

● *Original Contribution*

# HIGH FREQUENCY ULTRASOUND DEVICE TO INVESTIGATE THE ACOUSTIC PROPERTIES OF WHOLE BLOOD DURING COAGULATION

RACHEL LIBGOT-CALLÉ,* FRÉDÉRIC OSSANT,*‡ YVES GRUEL,† PATRICK LERMUSIAUX,‡ and FRÉDÉRIC PATAT*‡

*Université François Rabelais Tours, Laboratoire Ultrasons Signaux Instrumentation, CNRS FRE 2448, Tours, France; †Department of Hematology Haemostasis, Inserm U618, Tours, France; and ‡University Hospital, Tours, France

(*Received* 31 *July* 2006; *revised* 8 *June* 2007; *in final form* 20 *June* 2007)

**Abstract—This study was designed to investigate the changes in acoustic properties of whole blood during the coagulation process. High frequency (from 20 to 40 MHz) ultrasound parameters were measured both in double transmission (DT) and backscattering (BS) mode to assess sound velocity and backscatter coefficient, respectively. The integrated backscatter coefficient (IBC) and the effective scatterer size (ESS) were deducted from the backscatter coefficient. Measurements were performed on whole blood samples collected from 12 healthy volunteers. During the blood clotting process (2 h observation), acoustic parameters were measured with 15 s time resolution for the transmission parameter and 5 s (for the 5 first min) and 30 s (for the end of the observation time) for the backscattering parameters. The results obtained clearly showed that simultaneous measurements of parameters in DT and BS modes are able to identify several stages during the *in vitro* blood clotting process. In particular, red blood cell (RBC) aggregation can be described from the backscattering parameters and liquid-gel transition phase of blood from the sound velocity. Intra- and inter-individual dispersion of these parameters were also measured and discussed. (E-mail: libgot_r@med.univ-tours.fr)** 

***Key Words:*** **Whole blood coagulation, Integrated backscatter coefficient, Effective scatterer size, Acoustic velocity, High frequency ultrasound.**

## INTRODUCTION

One of the main causes of mortality in the world nowadays is cardiovascular disease, *i.e.*, coronary and venous thrombosis, pulmonary embolism and atherosclerosis. A significant proportion of these diseases are the consequence of activation of whole blood coagulation and thrombosis. Better understanding of this process and early detection of blood activation and clot formation are, therefore, essential for adequate prevention and treatment of thrombosis. Most existing clinical tests such as prothrombin time (PT) and activated partial thromboplastin time (APTT) are performed on plasma containing all the coagulation factors necessary for normal thrombin generation. Such tests (Trzeciak and Denninger 2004) are mainly based on photograph-optical detection of the fibrin clot (plasma sample) or electromagnetic detection of the stopping of a steel ball inside the test tube (plasma or whole blood sample) or mechanical detection of the stopping of a plunger placed inside the test tube (whole blood sample), corresponding to clot formation. Few *in vitro* tests that are also used in clinical practice (Hett et al. 1995; Forestier et al. 2001; Luddington 2005) are performed on whole blood samples. They include whole blood clotting time, activated coagulation time (Hemochron®, Medtronic HemoTec®), Thromboelastograph® and Sonoclot®, which measures viscoelastic changes in clots and the platelet function analyzer (PFA-100™), which focuses on platelet aggregation and adhesion. To make a relevant diagnosis, it is often necessary to perform at least two of these tests. Clinicians have recently reconsidered the value of using whole blood samples rather than plasma samples in hemostasis tests, for instance to take into account the role of phospholipids on the RBC surface in the process. Moreover, considering hemostasis functions rather than one specific factor appears to provide more information. There is, therefore, a

Address correspondence to: Rachel Libgot-Callé, Université François Rabelais Tours, Laboratoire Ultrasons Signaux Instrumentation, CNRS FRE 2448 Tours, France. E-mail: libgot_r@med.univ-tours.fr

need to develop an accurate and global standard coagulation test using whole blood samples (Sorensen et al. 2002; Barrowcliffe et al. 2006; http://www.fda.gov/cdrh/meetings/coag.html).

Ultrasound techniques have the major advantage of being noninvasive and they describe the blood clotting process through nontransparent tissue or media, and hence provide real-time measurements *in vivo*. Nevertheless, the literature on the *in vitro* monitoring of the coagulation process with ultrasound is sparse. Yesner et al. (1951) first used 28 kHz ultrasound to assess the absolute viscosity of blood while clotting. About 20 y later, the many improvements in electronics and US transducer machining made it possible to estimate acoustic parameters more accurately. The main goal of the use of ultrasound techniques was to evaluate blood coagulation time, corresponding to fibrin formation. Techniques were based on assessment of the ultrasound backscattering (BS) intensity (Shung et al. 1975; Machado et al. 1991) or ultrasound speed (Jacobs et al. 1976; Barone and Das 1979; Grysbauskas et al. 1978; Voleisis et al. 2002) and the absorption coefficient (Grysbauskas et al. 1978), using either plasma or whole blood samples, in the frequency range 1 to 10 MHz. Qualitative *in vitro* (Sigel et al. 1980, 1984; Coelho et al. 1982; Peter et al. 1986) and *in vivo* (Fowlkes et al. 1998) echogenicity studies, based on monitoring A- or B-mode imaging using 3 and 10 MHz transducers were performed in the 1980s. More quantitative measurements appeared in the 1990s using ultrasound tissue characterization methods, based on the spectral analysis of RF echo signals. Most of these studies were performed specifically to study the red blood cell aggregation phenomenon without blood clotting. Such studies mainly involved ultrasound backscatter (Shung et al. 1976; Boynard et al. 1987; Mo et al. 1993; Cloutier and Qin 1997) and relative parameters such as scatterer size (Sigel et al. 1990). The slope and the y-intercept (Loiacono et al. 1992) and the integrated BS coefficient (IBC) (Recchia and Wickline 1993; Komiyama et al. 1999) were determined in the frequency range 5 to 30 MHz.

US techniques based on the simultaneous measurement of BS and transmission parameters were also suggested. Shung et al. (1984) proposed a system able to estimate both the BS and the attenuation coefficient and also the acoustic velocity in whole human blood at 7.5 MHz. After preliminary studies, we reported the first results of the simultaneous measurement of IBC, ESS and velocity during the human whole blood coagulation process in the frequency range 10 to 40 MHz (Ossant et al. 2004; Libgot et al. 2005). Huang et al. (2005) worked on the simultaneous evaluation of IBC, attenuation coefficient and acoustic velocity with 10 MHz transducers during the porcine whole blood coagulation process.

Other US techniques such as elastography (Genisson et al. 2004) and combination methods of elasticity imaging with US duplex (Emelianov et al. 2002) or with photoacoustic imaging (Karpiouk et al. 2005) were also proposed to follow the coagulation process using shear waves and age-induced thrombus *in vivo*. The use of Shear-Horizontal Surface Acoustic Wave (SH-SAW) sensors was also studied (Guhr et al. 2005).

The aim of the study reported here was to propose an *in vitro* method for analysis of the interaction of high frequency ultrasound (from 20 to 40 MHz) with human whole blood during the clotting process in static conditions. The choice of working in static conditions was determined by results obtained by several authors (Yuan and Shung 1988; Van der Heiden et al. 1995; Fatkin et al. 1997; Cloutier et al. 2000) on BS measurement from which they concluded that low flow or stasis leads to enhanced formation of red blood cell aggregates. The measurement method used in the present study was based on the simultaneous measurement of both double-transmission (DT) and backscattering (BS) ultrasound parameters. Such simultaneous measurement provided quantitative results of acoustic velocity, integrated backscatter coefficient (IBC) and effective scatterer size (ESS).

## MATERIALS AND METHODS

### *Sampling of Whole Blood and Preparation Protocol*

Two series of *ex vivo* experiments were performed using whole human blood. The first was designed to monitor the whole blood coagulation process and the second was performed to emphasize the RBC aggregation phenomenon and was conducted with nonrecalcified whole blood. Blood samples were obtained from healthy volunteers in compliance with the Ethics Committee of the University Hospital of Tours.

*Protocol to Study Whole Blood Coagulation.* Blood samples collected in 3.5 mL vacuum sodium citrate tubes (3.2%), previously heated to 37 ± 0.1°C in an incubator (model 200, Memmert, Schwabach, Germany). Tubes with whole blood were then kept at 37°C for 30 min, and coagulation was initiated by adding calcium chloride (100 μl at 0.5 mol/L concentration). Tubes were rocked back and forth for 30 s (Labquake® model 1419, Barnstead International, Dubuque, IA, USA) to disrupt RBC aggregates. Blood was then immediately transferred to the blood chamber of the cell and acoustic measurement began. All measurements were performed in static conditions in an incubator at 37 ± 0.1°C.

Hematocrit (Ht) and fibrinogen concentration (Fib.)